REMARKS

Claims 60-145 were previously pending in this application. Of those previously pending, claims 60-73 were elected claims and claims 74-145 were withdrawn from consideration as being drawn to a non-elected invention. New claims 146-200 have been added above in place of the elected claims, the latter (60-73), together with the non-elected claims (74-145) having been canceled hereinabove. Accordingly, claims 146-200 are being presented for further examination on the merits.

New Claims

Commensurate with Applicants' complete and broad disclosure, new claims 146-200 have been added above. Claims 146, 166 and 183 are independent processes for detecting the presence of a specific target nucleic acid sequence. Briefly, claims 146, 166 and 183 are directed to different aspects or embodiments as follows. In claim 146, the first step of providing 1) recites that synthesis and extension of the first segment of the first initial primers or first nucleic acid constructs with the specific target nucleic acid sequences as a template continues to be in the absence of a denaturation step. Claim 166 lacks such a recitation. Instead, claim 166 recites in the incubating step 3) that "extension is only carried out using first initial primers or nucleic acid constructs comprising said two segments." In claim 183, the second segment of the one or more first initial primers or first nucleic acid constructs is defined in part (B) as "being capable of participation in the formation of a stem-loop structure at one end of said first extended nucleic acid strand after said specific target nucleic acid sequence is used as a template for extension." In all three independent claims (146, 166 and 183), the formation of the stem-loop structure has been

better defined by the inclusion of a new step 4). Step 4 recites "forming at least one stem-loop structure by (a) self-annealing between said second segment of said first initial primer or first nucleic acid construct and a segment derived from target template dependent extension of the first segment of said first initial primer or first nucleic acid construct, and (b) separating said first segment of said first initial primer or first nucleic acid construct from said specific target nucleic acid sequence."

Many if not most of the dependent claims added above mimic the subject matter of former and now canceled dependent claims. In drafting new claims 146-200, attention was paid to the objection of the claims in the December 20, 2000 Office Action for improper multiple dependency (37 C.F.R. §1.75(c)).

Entry of claims 146-200 is respectfully requested.

New Group/or Art Unit

Acknowledgement is made that the Group and/or Art Unit for this application has been changed. Any and all future correspondence will be directed to Group Art Unit 1656.

Previous Restriction Requirement

Acknowledgement is also made that the restriction requirement set forth in the December 20, 2002 Office Action was made final. Applicants intend to pursue the subject matter of the previous non-elected claims in several duly filed divisional applications.

New Title

A new title of the invention has been added above in response to the Examiner's comments on page 5 in the December 20, 2000 Office Action. The new title ("Detecting The Presence Of Specific Target Nucleic Acid Sequences Through Stem-Loop Formation") is believed to be more descriptive of the subject matter being pursued in this divisional application.

The Objection Under 37 C.F.R. §1.75(c)

Claims 65-66, and 71-73 stand objected to under 37 CFR §1.75(c) as being in improper form because [a multiple dependent claims cannot depend from another multiple dependent claim]. See MPEP § 608.01(n). Accordingly, the claims 65-66, and 71-73 have not been further treated on the merits.

As discussed in the opening remarks of this Amendment, the objection to the claims is believed to have been obviated by the presentation of new claims 146-200 above. No multiple dependent claim is seen to depend from any other multiple dependent claim.

Reconsideration and withdrawal of the objection under 37 C.F.R. §1.75(c) is respectfully requested.

The Rejection Under 35 U.S.C. §112, Second Paragraph

Claims 60-73 are rejected under 35 U.S. C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In the Office Action (pages 6-7), the Examiner stated:

a. Claims 60-73 are vague and indefinite because of the language "substantially complementary to", "substantially identically", "substantially non-identical" in claim 60 which is unclear how the language with "substantially" is defined in the specification. In addition, the language "derived from" is unclear because the segment is synthesized by the extension of the first segment of the first primer from the target nucleic acid used as a template, and the segment is not a derivative with the chemical modification of the extension of the first segment of the first primer from the target nucleic acid used as a template. It is suggested to clarify uncertainty.

b. Claims 67-73 are vague and indefinite because of the language "substantially complementary" in claim 67 which is unclear how the language with "substantially" is defined in the specification. In addition, it is unclear which template is used to synthesize the sequences that are synthesized after extension of the first initial primer and is substantially complementary to the first segment of the second primer.

c. Claims 68 and 70-73 are vague and indefinite because of the language "substantially non-identical to", "substantially identical to" and "substantially complementary to" in claims 68 which is unclear how the language with "substantially" is defined in the specification.

d. Claim 72 is vague and indefinite because it is unclear how the language "substantially completed" is defined in the specification.

e. Claim 73 is vague and indefinite because it is unclear how the language "substantially completed" is defined in the specification. In addition, it is unclear how the detection is done by detecting the formation of a stem-loop structure which takes place before nucleic acid synthesis because as set forth in claim 60 the stem-loop structure is formed by self-annealing between the second segment of the first initial primer and a segment synthesized from the target used as template by the template dependent extension of the first segment of the first initial primer. It is suggested to clarify uncertainty.

f. Claims 67-73 are vague and indefinite because it is unclear how the second initial primer is involved in the detection of the specific target nucleic acid sequence with the detection of the presence of the stem-loop structure.

g. Claim 66 and 72-73 are vague and indefinite because it is unclear how the detection is taken place without the amplification of the target nucleic acid.

h. Claims 60-73 are vague and indefinite because it is unclear without a denaturation step how the first segment of the first initial primer is extended and the stem-loop structure is detected. It might mean that the process is one single cycle amplification. It is suggested to clarify uncertainty.

The indefiniteness rejection is respectfully traversed.

With respect to Points a through d above, it is respectfully submitted that the term "substantially" as recited in the claims is clear and definite, and altogether proper claim language under the law. In most instances, the new claims recite substantially with respect to the degree of complementarity, identity or reaction completion. It is believed that a person skilled in the art of nucleic acid synthesis, hybridization, detection or amplification would understand the meaning of "substantially" as it is applied to nucleic acid complementarity, non-complementarity, identity, non-identity and reaction completion. For example, a nucleic acid sequence which is substantially complementary to another nucleic acid sequence, would still be able to bind or hybridize to each other, despite the lack of total complementarity.

In addition to the remarks above, in regard to Point e in the rejection, Applicant respectfully further point out first, all that is required for stem-loop structure formation is synthesis of a segment that is complementary to the second segment of the first initial primer or first nucleic acid construct. Thus, stem-loop structure formation can take place prior to extension of further sequences. Second, Applicants respectfully point out that the ability to generate a stem-loop structure allows for further binding and extension events of other first initial primers or first nucleic acid constructs. These features are described in the specification. See, for example, Figure 1, first full paragraph ("In this way, in the absence of denaturing conditions, the novel process of the present invention can provide for multiple priming, extension and release events from a single strand of a nucleic acid template. Furthermore, all of these steps can take place simultaneously and continuously under isostatic conditions.").

With respect to Point f in the rejection, Applicants respectfully point out that their disclosure describes that the provision of a second initial primer or second nucleic acid construct leads or can lead to non-linear amplification. By so doing, the rate of

synthesis and conversion of primers or constructs to stem-loop structures is increased. Hence, signal generation is attained and enhanced.

Regarding Point G, it is believed that the presentation of new claims 153 and 154 obviate this ground of rejection. As described earlier in the opening remarks of this Amendment, new claim 153 recites "wherein said mixing step 2), the sample is the product of an amplification reaction." Claim 154 recites wherein said mixing step 2), the sample has not previously undergone amplification or has been subjected to an amplification process. The previous claims had referred to the specific target nucleic acid in the sample prior to carrying out the claimed process at hand. The new claims clarify the claimed subject matter by referring to the sample, and not the specific target nucleic acid in the sample.

Regarding Point h in the rejection, it is not entirely clear to Applicants what the connection is between the absence of a denaturation step and primer extension. With respect to extension of the first segment of the first initial primer or first nucleic acid construct, it is plain in the claim language that denaturation is not a necessary step for carrying out the recited extension step in 3). Single-stranded forms of nucleic acid are found in nature and are of use in the present invention as specific target nucleic acids. A notable example of such is messenger RNA (mRNA). It should be noted that a new dependent claim (147) has been added which specifically recites "wherein prior to or after said mixing step 2) any double-stranded nucleic acids in said sample have been rendered single-stranded. In addition, new claim 148 depends from claim 147 and it recites "wherein said single-stranded rendering has been carried out by heat denaturation."

The process of claim 146 does refer to the "absence of a denaturation step," but it is only within the context of being capable of forming a stem-loop

structure. Applicants' disclosure and their Figure 1 particularly point out that the formation of a stem-loop structure in the absence of a denaturation step. See the specification, page 21, first full paragraph; and Figure 1. For the purposes of clarifying Applicants' invention, however, claim 146 now recites "4) forming at least one stem-loop structure by (a) self-annealing between said second segment of said first initial primer or first nucleic acid construct and a segment derived from target template dependent extension of the first segment of said first initial primer or first nucleic acid construct, and (b) separating said first segment of said first initial primer or first nucleic acid construct from said specific target nucleic acid sequence."

Finally, in Point h, Applicants respectfully point out that the presence of a stem-loop structure provides for additional synthesis that is dependent upon such formation. This is but one means of determining that stem-loop formation has taken place.

In view of the presentation of the new claims and the foregoing remarks, Applicants respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. §112, second paragraph.

Commonality of Ownership

Applicants point out that the subject matter of any of the present claims was commonly owned at the time any invention was made.

The Rejection Under 35 U.S.C. §103

Claims 60-65 and 67-72 stand rejected as being unpatentable over Western et al., U.S. Patent No. 5,612,199. In the Office Action (pages 8-11), the Examiner stated:

Western et al. disclose a method of detecting the presence of a nucleic acid analyte by examining the presence of the extended primer (See column 6, lines 45-49 and column 7, lines 17). The method allows extension of an extended probe along a single stranded target sequence to produce a single stranded sequence having the capability of forming an intramolecularly based paired structure in which the 3' end not involved in the production is modified (See column 7, lines 55-64). The extended probe comprises the sequence EP1 at 3' end which is hybridized to the S1 sequence of the analyte and the other sequence EP2 is substantially identical to S2 sequence of the analyte and not complementary to the analyte (See column 6, lines 46-48). A polydeoxynucleotide primer hybridizes to a nucleotide sequence complementary to S2 (See column 6, lines 46-48). The method also involves polymerase (See column 12, lines 37) and deoxynucleoside triphosphate as needed for the amplification reaction (See column 7, lines 1-5). The disclosure indicates that there is no means for degrading the extender probe to form the polynucleotide (See column 6, lines 66 to column 7, line 1). The target sequence identified within the analyte has S1 and S2, and S1 and S2 are separated by at least ten bases, preferably at least 100, usually 200-10,000 (See column 11, lines 24-28). The extender probe is extended along the target sequence and the extended sequence forms a stem-loop structure (See column 14, lines 36-40). The label or reporter group is bound to a nucleic acid probe or primer for detection (See column 16, lines 10-36). The polydeoxynucleotide primer is single stranded containing Tend '^ '^ hybridized to the complementary sequence of S2 sequence (See column 11, lines 66-68 to column 12, lines 1-4) and can be extended along the extended probe to form a duplex comprising the extended primer (See column 14, lines 24-32). This suggests that the extended primer can also have the structure to form a stem-loop which has the same function as the second initial primer since the extender probe which can form a stem-loop structure is complementary to the extended polydeoxynucleotide primer (See column 7, lines 55-64). Extension in this fashion provides the requisite fidelity between the extended primer and Vr polynucleotide so that accurate detection of target as the second initial primer as claimed because both are polynucleotide and have two segments, for example, first segment is complementary to sequence that are synthesized after extension of the extender probe.

The teachings of Western et al. suggest the limitations of claims 60-65 and 67-72. Instant claims 60-65, and 67-72 are drawn to a process of detecting the presence of a specific target nucleic acid sequence involving two initial primers. The first initial primers comprise two

segments in which the first segment is complementary to a portion of the specific target nucleic acid and capable of template extension and the second segment is non-identical to the first segment, identical to a portion of the specific target nucleic acid sequence and complementary to the sequence that are synthesized by extension of the first segment of the first initial primer. The second initial primer comprises the same structure as the first initial primer. The detection is done by the presence of the stem-loop structure by self-annealing between the second segment of the first initial primer and the segment synthesized from the extension of the first segment. The process also involves nucleic acid polymerase as listed in claim 61, the detectably labeled primers in which the moieties and labels are listed in claims 62-64 and 69-71.

One of ordinary skill in the art would have been motivated to use the method of Western et al. because the method of Western et al. is used for detecting the presence of a polynucleotide analyte in a sample by examining the presence of the extended primer (See column 6, lines 5-67 to column 7, lines 1-17) in which the produced single stranded polynucleotide can have an intramolecularly base-paired structure and 3' end of the extender probe is modified and the method provides a highly convenient method of converting a polynucleotide sequence of interest to a target sequence having an intramolecularly base-paired structure while minimizing the number of reagents and steps required (See column 7, lines 55-67 and column 8, lines 1-6). In addition, extension of the polydeoxynucleotide primer provides the requisite fidelity between the extended primer and the polynucleotide so that accurate detection of target analytes can be achieved (See column 14, lines 32-34). Thus, an artisan of ordinary skill in the art based upon the teachings of Western et al. would have made the instant invention as claimed. It would have been prima facie obvious to carry out the process as claimed.

The obviousness rejection is respectfully traversed.

In response, Applicants respectfully point that Western's cited patent was incorporated by reference into the specification at hand, and was addressed as prior art, and differences between Western et al. and the present invention were

described at length. See, for example, pages 23 and 33 in the present specification.[1]

In addition to any remarks in the present specification, Applicants wish to point out the following. Although Western et al. show an extended single strand that has the potential for forming a stem-loop structure, such a structure was used for illustrative purposes only to demonstrate that the ends of their disclosed amplicons were self-complementary. In point of fact, the actual physical formation of a stem-loop structure is not a desirable result for Western's purposes, because it would clearly prevent the primers from binding to the ends of their extended single strands and using them as templates. Thus, Western et al. actually teaches away from Applicants' present invention in that the former's desired object (amplification) would be defeated in forming any such stem-loop structure.

The present invention does not share this problem or deficiency with Western et al. because the former uses the loop segments as primer binding sites, rather than the stem sequences which are required as primer binding sites by Western et al.

These patentable distinctions are neither disclosed, suggested or addressed by Western et al.

In view of the new claims and the foregoing remarks, Applicants respectfully request reconsideration and withdrawal of the obviousness rejection, thereby placing all of the claims under examination in condition for allowance.

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[1] It should be noted that Western's cited patent belongs to a family of patents, the latter of which is referred in Applicants' specification as "Rose et al."

SUMMARY AND CONCLUSIONS

Claims 146-200 have been added above in place of the former claims 60-73 which have been canceled.

The fee for adding new claims 146-200 is $558, based upon the presentation of 62 additional claims above the 59 claims previously paid for [62 X $9 = $558]. As indicated in the accompanying Transmittal form, authorization is hereby given to charge the $558 claim fee amount to Deposit Account No. 05-1135. No other fee or fees are believed due in connection with this filing. In the event that any other fee or fees are due, however, The Patent and Trademark Office is hereby authorized to charge the amount of any such fee or fees to Deposit Account No. 05-1135, or to credit any overpayment thereto.

If a telephone conversation would further the prosecution of the present application, Applicants' undersigned attorney request that he be contacted at the number provided below.

Respectfully submitted,

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